ELECTROCHEMICAL CHARACTERIZATION OF CADMIUM-BINDING PROPERTIES OF METALLOTHIONEIN

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Abstract

A voltammetric study on the binding properties of the metallothionein (MT) for cadmium ions was performed. The capacity of metallothionein to complex cadmium and the stability constants of cadmium-thionein complex have been determined from the direct titration of MT with cadmium ions in model seawater medium at pH 7.9. At this pH the formation of the Cd-T complex has been followed measuring the specific anodic signal height of the complex. The apparent concentration stability constants of Cd-T complex, $K'=(7.6\pm0.2)\cdot10^8$ $dm^3\ mol^{-1}$, in 0.59 M NaCl, pH=7.9, 25°C, have been evaluated from the experimental data using three different procedures.

Key-words: cadmium, electrochemistry

Introduction

Metallotioneins (MTs) are low molar mass (6000 to 7000 for the protein of mammalian origin) metal-binding proteins known to occur in all animal phyla as well as in fungi, in some plants and cyanobacteria (1). The protein was designated as "metallothionein" on account of its exceptionally high metal and sulphur content (2). SH-groups involved in the metal coordination are considered essential to the structure and the function of the protein. MTs induced in the hepatic tissues are specific molecular biomarkers of the exposure of vertebrate and invertebrate species to metals (3,4). They can be induced by and bind the essential metals copper and zinc, and toxic metals such as cadmium, mercury and silver. MTs of mammalian origin are characterized in detail regarding their biological and physico-chemical features, the binding sites, stoichiometry and geometry of metal complexes (1). To the mammalian protein 7 atoms of Cd(II) are bound per MT molecule (Cd7-MT), and metal is tetrahedrally coordinated in two isolated domains with stoichiometries of M_4S_{11} and M_3S_9 (5).

The induction of MTs in *Mytilus* sp. has been determined in both laboratory and field studies (6-10). In contrast to the mammalian type of MTs, the mussel type of MTs is insufficiently characterized. It has recently been published (11) that the mussel MTs consist of seven isoforms and exhibit more similarity to the vertebrate MTs than to those of non-molluscan invertebrates. Isoforms of mussel MTs exhibit homology to the mammalian class I MTs. Therefore, a preliminary study on cadmium binding properties of commercially available well defined MTs of mammalian origin has been performed in order to evaluate the experimental conditions and the procedure suitable for subsequently studying cadmium binding properties of MTs isolated from the *Mytilus* sp. which are insufficiently characterized (12, 13). The purpose of providing complexing data on this specific inducible type of protein with Cd^{2+} is to gain a better understanding of the biological role of MTs during metabolism and detoxification of cadmium ions.

Experimental

A simple model seawater medium, *i.e.* 0.59 M NaCl solution was used in order to reduce the competitive reactions with Cd^{2+} as would occur in a complex medium of genuine seawater (14). The pH of model seawater was kept at pH 7.9 with the borate buffer. When needed, the model seawater was acidified to pH<2 with the destiled HNO_3. The standard Cd^{2+} solution (1.000±0.002 g/l) was prepared from the Titrisol solution (Merck, Germany), which contains $CdCl_2$. Metallothionein (MT I+II rabbit liver M7641) was produced by Sigma (USA). All solutions were prepared with Milli-Q water.

The voltammetric technique has been applied due to its sensitivity and selectivity in determining metal content and various chemical forms. The measurements were carried out with a μAutolab instrument (Eco Chemie, The Netherlands). Voltammetric measurements were performed under potentiostatic control with a three-electrode system consisting of a Metrohm 290E hanging mercury drop electrode (HMDE) as a working electrode, a platinum wire as a counter-electrode and a reference Ag/AgCl-saturated KCl electrode, which was connected to the cell by a salt bridge filled with 0.59 M NaCl (suprapure, Merck) in a 20 ml Metrohm-type polarographic cell. Measurement parameters set up for differential pulse anodic stripping voltammetry (DPASV) were the following: deposition potential -0.9 V vs. the potential of reference electrode, deposition time 120 s, resting time 30 s, pulse amplitude 25 mV, pulse duration 57 ms, scan rate 5 mV/s and clock time 0.5 s. All measurements were performed at constant temperature of (25.0±0.5)°C.

Results

Titrations of MT Sigma M7641 have been carried out with a standard $CdCl_2$ solution at pH=7.9 and pH<2 in a well defined electrolyte (0.59 M NaCl). At pH=7.9 two distinct anodic signals which represent two types of cadmium species (12, 13) are observed on the voltammograms. One type corresponds to the cadmium-thionein complex (Cd-T - where T denotes the apoprotein molecule), with the peak potential at -0.67 V vs. Ag/AgCl reference electrode (Fig. 1).

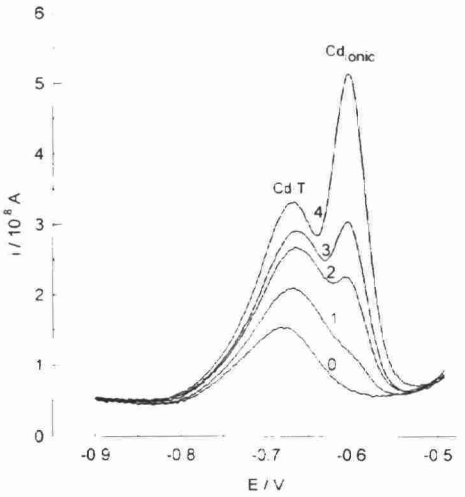

Fig. 1. Current-potential curves of $3.2\cdot10^{-8}$ M MT in 0.59 M NaCl at pH=7.9 (curve 0). Concentration of added $CdCl_2$ was the following: $7.10\cdot10^{-9}$ M Cd(II) (curve 1); $1.06\cdot10^{-8}$ M Cd(II) (curve 2); $1.42\cdot10^{-8}$ M Cd(II) (curve 3); $2.48\cdot10^{-8}$ M Cd(II) (curve 4). Two types of Cd(II) species are denoted as Cd-T complex (where T denotes the apoprotein molecule) and Cd_{ionic}.

The other one corresponds to the Cd_{ionic} (comprising hydrated Cd^{2+} ions and Cd(II)-chloro complexes), with the peak potential at -0.60 V vs. Ag/AgCl reference electrode (Fig. 1). The formation of the Cd-T complex has been directly followed at pH=7.9 measuring the anodic signal height of the Cd-T complex. Concentrations of Cd-T complex and Cd_{ionic} were calculated according to the slope of the calibration straight-line for cadmium under three different conditions:

(I) at pH=7.9 without the addition of MT;

(II) at pH<2 without the addition of MT;

(III) at pH<2 with the addition of $3.2\cdot10^{-8}$ M MT.

After the addition of MT at pH<2 only one signal of Cd(II) is visible at -0.60 V vs. Ag/AgCl, which corresponds to the ionic Cd(II) form (Fig. 2).

According to Ruzic (15), plotting $[M]_{ionic}/(M_T\text{-}[M]_{ionic})$ vs. $[M]_{ionic}$, a straight line should be obtained if one type of complex is predominant (MT is the total metal concentration and $[M]_{ionic}$ is the concentration of the labile metal species). From the slope the metal-